Title: **Acute 96-h Toxicity Tests**

Author: _____ Date: _____
Pete Key

Program Manager: _____ Date: _____
Michael H. Fulton

Branch Chief: _____ Date: _____
Geoffrey I. Scott

1.0 OBJECTIVE

To conduct an acute, 96-h toxicity test.

2.0 HEALTH AND SAFETY

Personnel should wear lab coats, safety goggles and chemical resistant gloves when preparing chemical stocks, and when dosing with test chemicals or effluents.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

This method should be restricted to use by or under the supervision of professionals experienced in aquatic toxicity testing.

4.0 REQUIRED AND RECOMMENDED MATERIALS

This section lists the required supplies and equipment:

Environmental chamber
Light meter
Pipette bulbs
Serological pipettes
pH meter
Thermometer
Media containers

5.0 PROCEDURE

5.1 Grass Shrimp 96-h Toxicity Test – Static-Renewal

1. Acclimate adult grass shrimp, 20 – 30 mm in length, for at least 14 days after collection. Shrimp with gill plate parasites or gravid females should not be used for the test.

2. Prepare test material stock according to SOP.

3. If a water-miscible organic solvent, such as acetone, is used to prepare the stock, the concentration of solvent should be the same in all test solutions including control. Do not exceed 0.1% acetone per treatment and control.

4. If Deionized Water is also used in the stock, the concentration of DI water should be the same in all test solutions including control. If stock is 100% organic solvent, do not add DI.

5. In order to allow calculation of an LC50, EC50 or IC50, the test concentrations should bracket the predicted concentration. If a prediction is not available, a range-finding test should be conducted.

6. For the test, there should at least be one control and a geometric series of five concentrations of test material. Each concentration should be at least 50% of the next higher one. Two or more replicates are desirable.

7. The percentage of grass shrimp in the control that show signs of disease or stress or death must be 10% or less.

8. Conduct all tests in an environmental chamber at 25°C
16-h light:8-h dark cycle
20 ‰ salinity
60 to 100% D.O. saturation
8.0 pH

9. Exposure containers: 1 gallon, glass, wide-mouth jars with screw on plastic lid with a Teflon liner. The lid should have a hole drilled in the center to just fit a 1 ml glass pipette. which is inserted to within 2 inches of the bottom of the jar to aerate the media.

10. Amount of test solution: 2-L per jar

11. Test Concentrations: Make up test concentrations in a glass vessel (i.e. Erlenmeyer flask, cylinder, beaker, etc.). Start with lowest concentration and work up to the highest. Add half amount of seawater needed to the vessel. Add solvent and test material stock according to predetermined calculations (SOP). Mix well by stirring or swirling vessel. Add remaining seawater. Mix again. Decant desired amount into exposure containers. After all concentrations have been made, rinse mixing vessel with acetone. Control test solutions should be made the same way but in a separate vessel.

12. Number of grass shrimp: 10 shrimp per jar (200 ml test solution per shrimp). After all test concentrations have been prepared, randomly add shrimp, 3 or 4 at a time, to each jar until each jar contains 10 shrimp.

13. Aeration: An airline will be attached to the end of a 1 ml glass pipette and inserted into the lid of each jar. To each pipette, attach tape approximately 2.5 cm from the airline to keep pipette within 2 inches of the bottom of the jar. Adjust air flow to maintain at least 60% saturation – a calm, steady stream of bubbles will suffice.

14. Placement of test containers: Place in environmental chamber in a random pattern. Use a random number generator if necessary.

15. Parameters: At 0-h and every 24-h thereafter, check temperature, salinity, DO (mg/L), and pH in all control jars.

16. After setup, the test materials will be changed out completely at 24, 48 and 72 h.
    a. Allow test material stock to warm to room temperature.
    b. Remove all containers from the environmental chamber.
    c. Check parameters as in step 15 and record.
    d. Check each concentration and control for mortality. Remove dead shrimp, place in a sealed container and freeze. Record number alive, dead and missing.
    e. Make up control water as in Step 11.
    f. Holding a net over a bucket, pour control containers through net to empty the container and catch the shrimp. Make sure that shrimp do not jump out of net. Pour fresh control water into container and return shrimp to container. Count shrimp in container to make sure that all have returned.
    g. Take test material mixing vessel and rinse well with seawater to remove any acetone that may be present after previous acetone rinse (Step 11).

Starting with the lowest concentration, make up test solution (Step 11). Pour out as in Step 16-f for each concentration starting with lowest and continuing through to the highest. Make sure to count shrimp after returning them to the container.

h. Spent control water may placed down the drain flushing well.

i. Spent test material concentrations will be placed in an appropriate storage container. Log in amount and concentration of test material in storage container. Storage container will be labeled with name of test material and date.

j. Return all test containers to the environmental chamber.

k. Do not feed adult shrimp during the 96-h test.

17. Observations of the test should be made other than the 24-h time periods. This is especially important with shrimp as cannibalism of dead shrimp is common.

18. Modifications to this SOP may be necessary as dictated by extenuating circumstances.

19. For further information, see ASTM, E 729-96, 1996, pages 1 - 21.

5.2 Mummichog 96-h Toxicity Test – Static-Renewal

1. Mummichog 96-h static renewal toxicity test will be performed the same as the grass shrimp toxicity test above except for the following changes:

a. Amount of test solution: 3.5-L per jar

b. Number of mummichog: 5 mummichog (45 – 75 mm in length) per jar (700 ml test solution per fish). After all test concentrations have been prepared, randomly add fish, 1 at a time, to each jar until each jar contains 5 fish.

2. For further information, see ASTM, E 729-96, 1996, pages 1 - 21.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Personnel should adhere to good laboratory practices performing this assay. This procedure should always be performed with proper precautions to minimize personnel exposure to toxic compounds.

7.0 REFERENCES

ASTM (American Society for Testing and Materials) (1996) Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates and amphibians, E 729-96 , West Conshohocken, PA 21 pp.

DeWoskin, R.S. 1984. Good laboratory practice regulations: a comparison. Research Triangle Institute, Research Triangle Park, North Carolina. 63 pp.

USEPA. 1979. Good laboratory practice standards for health effects. Part 772 - Standards for development of test data. Fed. Reg. 44:27362-27375, May 9, 1979.

USEPA. 1980. Physical, chemical, persistence, and ecological effects testing; good laboratory practice standards (proposed rule). 40 CFR 772, Fed. Reg. 45:77353-77365. November 21, 1980.